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THE RESPIRATORY QUOTIENT OF DROSOPHILA IN FLIGHT

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Introduction

In view of the unusual performance of insect flight muscles, which are able at times to make over a million successive contractions at rates up to several hundred per second, information on the biochemical transformations which supply the energy for this intense activity is of special interest. The purpose of this study has been to ascertain what evidence as to the types of substrate utilized during flight could be obtained from continuous measurements of the respiratory quotient.

The earlier students of the respiration of flying insects, whose results have been reviewed in detail by Jongbloed and Wiersma (1934), were generally content to assume a respiratory quotient of 1. Jongbloed and Wiersma were the first to provide an experimental basis for this theory when they allowed individual bees to fly for periods of about 5 minutes in closed containers and showed that oxygen was consumed and carbon dioxide given off in nearly equal amounts. Beutler (1936 a, b; 1937) then demonstrated that bees and some other Hymenoptera were dependent upon sugar for the ability to fly, and in fact for the maintenance of life, but because of the specialized physiology of bees and their adaptation to the food stores of the hive one is hesitant to transfer these findings without further support to other types of insects.

Nevertheless there is evidence that in the Diptera also the flight respiratory quotient is 1. Thus, Chadwick and Gilmour (1940) in two short flights of 5 and 6 minutes duration found with *Drosophila repleta* an equivalence of oxygen uptake and carbon dioxide output, while Williams and colleagues (1943) were able to show for *D. funebris* and *Lucilia sericata* a quantitative correspondence over a period of 60 to 90 minutes between the rate of disappearance of glycogen and the amount of flight activity as measured by the rate of wing-beat and the duration of flight. Whether carbohydrate was the only fuel consumed during these longer flights was not determined. Studies of flight respiration by Davis and Fraenkel (1940) and by Krogh and Zeuthen (1941), the most recent known to the writer, were concerned primarily with other aspects of the problem, and do not afford a basis for answering this question. Therefore, since the technique of Fenn (1928) provides a means of making continuous simultaneous measurements of oxygen consumption and carbon

* These experiments were done while the writer was a member of the Department of Physiology of the University of Rochester School of Medicine and Dentistry.

dioxide production, it seemed worthwhile to combine this method with stroboscopic determinations of the rate of wing-beat in order to study possible fluctuations in the respiratory quotient as related to activity, particularly in the later stages of prolonged flights.

Material and Method

The procedure followed differed little from that described by Chadwick and Gilmour, except that the Warburg manometers of their experiments were replaced by a differential volumeter with side tube for conductivity measurements, of the type devised by Fenn. The volume of each vessel was approximately 30 ml.; the capillary was 30 cm. long and had a capacity of about 5 cu.mm. per cm. CO_2 given off by the insect was absorbed in 3 ml. of 0.01–0.02 M $Ba(OH)_2$, which covered the bottom of the experimental vessel and was tipped into the side tube for measurement of the change in impedance at intervals of 1 minute while flight was in progress. Simultaneous records of O_2-consumption were obtained from the movement of an index drop in the capillary which connected the control and experimental vessels, while wing-beat frequency was measured stroboscopically, as described previously, at 10-second intervals.

The sensitivity of the measurements is estimated as follows:

CO_2	: $\pm$ 0.02 cu.mm.	(= 1 scale division on slide wire of conductivity bridge)
O_2	: $\pm$ 0.025 cu.mm.	(= 0.1 mm. on capillary scale read with aid of a magnifying glass)
wing-rate	: $\pm$ 1 per cent	(manufacturer's specification for stroboscope)

Relatively greater errors were introduced by imperfect synchrony between the three types of measurement, since, for example, a lag of 30 seconds between recordings of oxygen and carbon dioxide would place the second observation in excess by 50 per cent of the respiratory rate during the last minute of the period of measurement. The observers gave special attention to eliminating this source of error in so far as possible, so that when the measured amounts of oxygen or carbon dioxide have been summed for periods of 5 or more minutes, the uncertainty from this cause may be estimated conservatively at not more than 2 per cent of the totals.

It is more difficult to take account of lag in the absorption of carbon dioxide under the conditions of the experiments, where an insect of not more than 1.5 mg. weight in a vessel of 30 ml. capacity was using oxygen and producing carbon dioxide at variable rates up to nearly 1 cu.mm per minute. Absorption curves were determined for the vessel and showed that when a few cu.mm. of CO_2 were introduced 93–95 per cent was absorbed in 5 minutes, but these could give only an approximation to the actual experiments, the purpose of which was to follow continuously the unknown and unpredictably changing rate of gaseous exchange of the animal. Since the rate of absorption will be proportional at any instant to the concentration of gas existing in the vessel, identical quantities of carbon dioxide liberated over a given interval of time will give different percentages of absorption by the end of the period, if the lag in absorption is appreciable, depending on whether the rate of liberation was constant, rising or falling. Thus one would need independent knowledge of the rate of change in rate of CO_2-production in order to

apply satisfactorily any absorption "constants" which had been determined for the system. For this reason, no attempt has been made to correct the data presented below.

Lag in absorption of carbon dioxide not only introduces an asynchrony between the record of respiratory exchange and the measurements of wing-beat frequency when sudden changes occur, but may result also in spurious values for the respiratory quotient. Consider, for instance, the consumption of 100 units of oxygen and the production of 90 units of carbon dioxide during an interval in which only 70 per cent of the carbon dioxide produced is absorbed. Readings taken at the beginning and end of this period would indicate a carbon dioxide production of only 63 units; the remaining 27 units of this gas are still in the vessel, however, and occupy space vacated by a similar number of units of oxygen. Consequently the volumeter records the disappearance of only 73 units of oxygen. The apparent respiratory quotient for the period will therefore be given by the ratio 63:73 (= 0.86), whereas the true value is 0.90. Thus, the apparent respiratory quotient is depressed below the true value, if this is less than 1, and elevated above it, if it is really greater than 1. In many experiments lag in absorption will not lead to much error in the estimation of the respiratory quotient, since, if the rate of production of CO_2 remains constant, the concentration of the gas in the vessel and consequently the rate of absorption will rise, so that in course of time the apparent respiratory quotient will approach the true value. But in experiments with insect flight, it is often impossible to allow time for equilibration after changes in the rate of production of CO_2.

Evidently, if there had been no other factors to be considered, it would have been preferable in this study to have used vessels small enough to make the lag in absorption of CO_2 insignificant. But to have done so would have required rather drastic changes, which have not yet proved feasible, in the technique of the conductivity measurements. The data obtained with the larger vessel are therefore presented here and are to be interpreted with appropriate reservations, especially for periods in which marked changes occurred in the respiratory rate, such as at the beginning and end of flight.

As specimens for the study, adult males of known age were taken from cultures of *D. virilis* and *D. americana*, which were kindly supplied by Dr. H. D. Stalker. The animals were reared in the laboratory (temperature 20°–25° C.) in half-pint bottles on a standard medium. In preparation, the flies were immobilized with ether and the dorsal tip of the abdomen fastened with paraffin to a paper mount which was later affixed to the stopper of the respirometer vessel. Only one specimen was run at a time. The animal rested in the head-down position in the vessel, with the feet in contact with a light spring platform which could be retracted by means of an electromagnet situated outside the water bath in which the respirometer was immersed. Control measurements were made in order to ensure that the respiration recorded was that of the animals rather than the apparatus.

All experiments were run at a bath temperature of $20.0 \pm 0.01°$ C. After a half-hour for equilibration, the resting respiratory exchange was followed for a considerable length of time (see Table I) before the animal was stimulated to fly by withdrawing the platform on which his feet were supported. Flight continued until it ceased spontaneously, after which the resting exchange was again measured

for a number of hours during the post-flight period. The animal was weighed at the conclusion of the experiment.

These experiments would not have been possible without the interest and co-operation of Professor Wallace O. Fenn, in whose laboratory they were carried out. I am also indebted to my wife for assistance with the measurements.

TABLE I

Preflight respiratory exchange in Drosophila

Specimen number	Weight	Age	Duration of measurements	Average CO_2-output	Average O_2-uptake	Average R.Q.
	mg.	days	min.	cu.mm./gm./min.		
1	1.5	0–2	120	39.7	26.8	1.48
2	0.9	2	188	31.5	25.1	1.25
3	0.9	2	71.5	48.5	32.2	1.51
4	1.0	2	148	40.0	29.2	1.37
5	1.0	5	1460	20.6	27.6	0.75
6	0.7	2	253	35.6	32.1	1.11
7	1.1	2	102	33.4	28.7	1.16
8	0.9	2	181	30.8	19.9	1.55
9	1.5	2	380	23.2	18.8	1.23
10	1.2	3	369	28.6	23.1	1.24
11	1.4	2	294	28.1	25.0	1.13
12	1.1	5	367	25.3	18.2	1.39
13	1.5	7	369	20.8	22.1	0.94
14	1.2	2	191	37.2	35.2	1.06
Average				31.7	26.0	1.23

Specimens numbered 12, 13 and 14 were males of *D. virilis;* the others, males of *D. americana*.

RESULTS

a. Respiration before flight

Data on the respiratory exchange during the period before flight are given in Table I, in which the age and weight of the specimens are also recorded.

The average rate of oxygen consumption (26.0 cu.mm. per gm. per min.) is in good agreement with resting values which had been determined for *Drosophila* by other methods (Kucera, 1934; Chadwick and Gilmour, 1940). Comparable figures for production of CO_2 seem not to have been published previously.

b. Respiration during flight

Of the 14 flies listed in Table I, 6 flew continuously for periods of 56 to 154 minutes. The respiratory data obtained have been summed for each of these individuals over successive intervals of approximately 20 minutes, and are shown together with the corresponding average rates of wing-beat and the calculated respiratory quotients in Table II. The correlation between rates of wing beat and of respiration is illustrated graphically for 4 of these animals in Figure 1, where each point plotted represents approximately 5 minutes of flight. The average rate of oxygen consumption during these long flights was about 14 times, and of CO_2 production about 11 times the previous resting rate; in the earlier study with *D. repleta* the average oxygen consumption during flight was 13 times the value at rest.

TABLE II

Respiratory exchange of Drosophila in successive periods of longer flights

Specimen number	Duration of measurements min.	Average frequency beats/min.	Total CO_2-output cu. mm.	Total O_2-uptake cu. mm.	Average R.Q.
1	22	9208	17.14	16.09	1.06
	22	9125	16.39	17.68	0.93
	21	8669	13.75	14.34	0.96
	22	6927	8.82	9.31	0.95
3	21.5	9233	8.21	7.17	1.14
	20	7892	5.81	5.82	1.00
	20	7580	5.82	5.61	1.04
	9.3	7004	1.95	2.32	0.84
10	20.3	9345	14.48	13.89	1.04
	20	8721	11.67	11.36	1.03
	22	7860	10.68	9.98	1.07
	21.3	7827	10.13	10.45	0.97
	21	7729	9.29	9.77	0.95
	20.5	8065	10.88	11.03	0.99
	20.2	8181	11.10	11.69	0.95
	8.8	8201	4.97	4.66	1.07
11	19.0	9111	11.60	11.92	0.97
	22.3	8219	12.60	12.41	1.01
	18.7	7071	7.10	7.79	0.90
	22.2	6756	7.95	8.09	0.98
	20.2	6519	7.41	7.56	0.98
	22.7	6638	7.14	8.06	0.89
	21.5	6354	6.19	6.30	0.98
13	20.2	7659	8.83	8.96	0.98
	17.5	7450	8.09	7.30	1.11
	18.0	6917	6.48	6.09	1.06
14	21.0	8289	9.90	10.29	0.96
	17.3	7128	8.59	8.69	0.99
	13.2	7869	6.52	6.78	0.96
	12.8	7632	5.87	5.94	0.99

Performance in shorter flights is depicted adequately by the data for the initial periods of those flies which flew for longer times, and was covered also in the earlier publication.

c. Respiration after flight

Irregularly spaced measurements of oxygen consumption and carbon dioxide production after flight were made on the 6 individuals which flew for long periods. These have been recorded in Table III. Differences between the rates determined after flight and the average rates before flight are shown in columns 5 and 6 of the table.

TABLE III

Respiratory exchange of Drosophila after long flights

Specimen number	Interval of measurement	Rate after flight		Excess over rate before flight		Postflight R.Q.
		CO_2	O_2	CO_2	O_2	
	min.	cu.mm./gm./min.		cu.mm./gm./min.		
1	0–5	75	128	35	101	0.58
	5–10	39	36	−1	9	1.07
	10–30	25	41	−15	14	0.60
	30–50	23	31	−17	4	0.76
	50–92	22	45	−18	18	0.47
	92–1065	17	31	−23	4	0.56
	1065–1133	17	38	−23	11	0.46
	1133–1199	15	21	−25	−6	0.72*
	1199–1231	16	16	−24	−11	1.00*
3	0–4.7	161	117	112	85	1.39
	4.7–25.7	41	77	−8	45	0.54
	25.7–52.7	17	31	−32	−1	0.57
10	0–5	145	140	116	117	1.04
	5–14	53	89	24	66	0.60
	14–20	42	58	13	35	0.72
	20–31	34	36	5	13	0.96
	31–787	19	28	−10	5	0.66
11	0–13	66	83	38	60	0.79
	13–23	33	29	5	4	1.12
	23–43	25	24	−3	−1	1.04
	43–59	26	25	−2	0	1.04
	59–70	23	30	−5	5	0.76
13	0–8.5	110	85	89	63	1.28
	8.5–20.5	53	43	32	21	1.22
	20.5–37.5	32	29	11	7	1.11
14	0–8.3	92	87	55	52	1.06
	8.3–22.7	44	42	7	7	1.06
	22.7–50.2	32	39	−5	4	0.83
	50.2–75.2	34	38	−3	3	0.90

* Specimen moribund.

Rates are given to the nearest whole number; R.Q. computed from actual observations.

DISCUSSION

a. Respiration before flight

The most striking feature of the preflight respiration was the high respiratory quotient, which averaged less than 1 with only 2 of the 14 individuals examined (Table I). So far as could be determined, these observations were not the result of any artefact in the experiments and at first it was thought possible that the flies might be giving off ammonia as an end product of protein breakdown. If this should occur, the volumetric determinations of oxygen consumed would yield er-

roneously low values, while there would be no interference with the absorption of CO_2. The possibility was ruled out in several ways. In some experiments (numbers 12, 13 and 14 of Table I) an acid-soaked piece of filter paper was suspended in the experimental vessel, but high respiratory quotients were found throughout most of the period nevertheless. In other experiments, individual flies were confined over acid, which was then tested with Nessler's reagent for the presence of ammonia. A few early trials gave some positive results, but later when more care was taken to exclude contamination from outside sources, even large numbers of flies failed to produce significant amounts of ammonia. The high respiratory quotients were characteristic only of the first three hours or so of measurement, and fell to values of 1 or somewhat less when the observations were carried over longer periods.

While no definite explanation can be given for these facts, which deserve further study, it is suggested that the flies, recently removed from food in the culture bottles, may have been laying down energy reserves by conversion of carbohydrate to fat. Jongbloed and Wiersma (1934) occasionally noted high respiratory quotients before flight in their experiments with bees, but were inclined to ascribe these aberrant values to experimental error.

b. Respiration and rate of wing-beat during flight

Two objectives were in mind during this phase of the experiments: (1) measurement of the respiratory exchange, and (2) a retesting of the correlation found in a previous study between rate of wing-beat and the level of the respiratory metabolism.

As may be seen from the data in Table II, very seldom did the observations indicate a respiratory quotient significantly different from 1, even in the later stages of continuous flights which lasted from 1 to 2 or more hours. Thus the conclusion reached by Williams and colleagues on the basis of glycogen determinations, that carbohydrate constitutes the chief source of the energy required for flight, receives strong support from the present observations. These workers found that 4 to 5 day old *D. funebris* when freshly removed from culture contained glycogen to the extent of 4.88 per cent of the live weight. After 90 minutes of flight only 1.30 per cent remained, so that its rate of disappearance, in terms of the weight of the animal, amounted to about 2.4 per cent per hour. Very similar results were obtained from analyses of thoracic glycogen in the blowfly, *Lucilia sericata*.

For comparison, the weights of glycogen equivalent to the CO_2 which was produced in the flights of the present study have been calculated. It was assumed that complete oxidation of the carbohydrate to CO_2 and water occurred, on which basis the rate of utilization of glycogen during flight amounted to from 2.0 to 3.2 per cent of the final live weight per hour (Table IV). These figures are sufficiently close to those obtained by Williams *et al.* to make it seem likely that glycogen is not merely the principal, but probably the sole source of energy consumed during flight; however, the scatter in the results is such as not to exclude the possibility that smaller amounts of other types of substrate might also have been utilized, although there is no definite evidence pointing in this direction.

An intimate correspondence between the level of activity and the respiratory rate is brought out in the plots of Figure 1, where the logarithms of the rates of CO_2-production are compared with the logarithms of the rates of wing-beat. Each

point represents the average rates over a period of approximately 5 minutes, and the rate of production of CO_2 has been corrected by subtraction of the average rate found for the specimen in the preflight resting period.

TABLE IV

Glycogen equivalents of respiration of Drosophila during long flights

Specimen number	Weight	Duration of flight	Total wing-beats (approximate)	Total CO_2 produced		Calculated consumption of glycogen	
	mg.	min.		cu. mm.	micrograms	micrograms	per cent of body weight per hour
1	1.5	87	738,000	56.10	110.0	67.5	3.2
3	0.9	71	573,000	21.79	42.7	26.2	2.4
10	1.2	154	1,268,000	83.20	163.0	100.0	3.2
11	1.4	147	1,064,000	60.00	117.6	72.2	2.1
13	1.5	56	409,000	23.40	46.0	28.2	2.0
14	1.2	64	484,000	31.29	61.5	37.7	2.9

Inspection of the graphs shows clearly that there must be some underlying linear relationship between the logarithms of frequency and respiratory rate. Similar results had been obtained during shorter flights in the majority of the experiments of Chadwick and Gilmour, who reasoned from a comparison of wing movement with simple harmonic motion that the work done per stroke should be proportional to the (frequency)^2, so that a direct proportionality between (frequency)^2 and the oxygen consumption per stroke would be expected. Essentially the same relationship was developed from a somewhat different approach by Reed *et al.* (1942) in a study in which the characteristic rate of wing-beat under standard conditions was compared with various bodily dimensions for a number of species and races of *Drosophila*. The formula they derived,

$$(\text{wing-beat frequency})^2 = k \times \text{muscle volume}/(\text{wing area})\ (\text{wing length})^3,$$

expresses the fact that the (wing-beat frequency)^2 increases in direct proportion to the power of the motor mechanism and in inverse proportion to the resistance to be overcome by the wings.

If the work done per stroke is proportional to the second power of the frequency, then the work done in unit time must be proportional to (frequency)^3, and this quantity would be related directly to the rate of respiration, the energy input, through an efficiency factor. In the absence of satisfactory determinations of the work output of *Drosophila* in flight, the actual efficiency remains unknown, but the coefficient may be supposed to include, in addition to the overall efficiency of the biochemical transformations which supply the energy of muscular contraction, a second component expressing the efficiency with which the thorax and wings are able to translate muscular work into movement of air. A perfectly linear relationship between (frequency)^3 and rate of respiration would indicate that the efficiency remained absolutely constant throughout the period of flight, or that changes in efficiency were exactly balanced by shifts in amplitude or in the angle of attack.

As an aid in visualizing the actual performance, straight lines have been drawn through each of the plots in Figure 1 to conform to the equation

$$\log\ (CO_2/t - r) + \log\ k = 3\ \log\ F, \qquad (1)$$

where CO_2 = total CO_2 recorded during time t,
r = mean rate of production of CO_2 during the preflight resting period, and
F = average frequency during time t in wing-beats per minute.

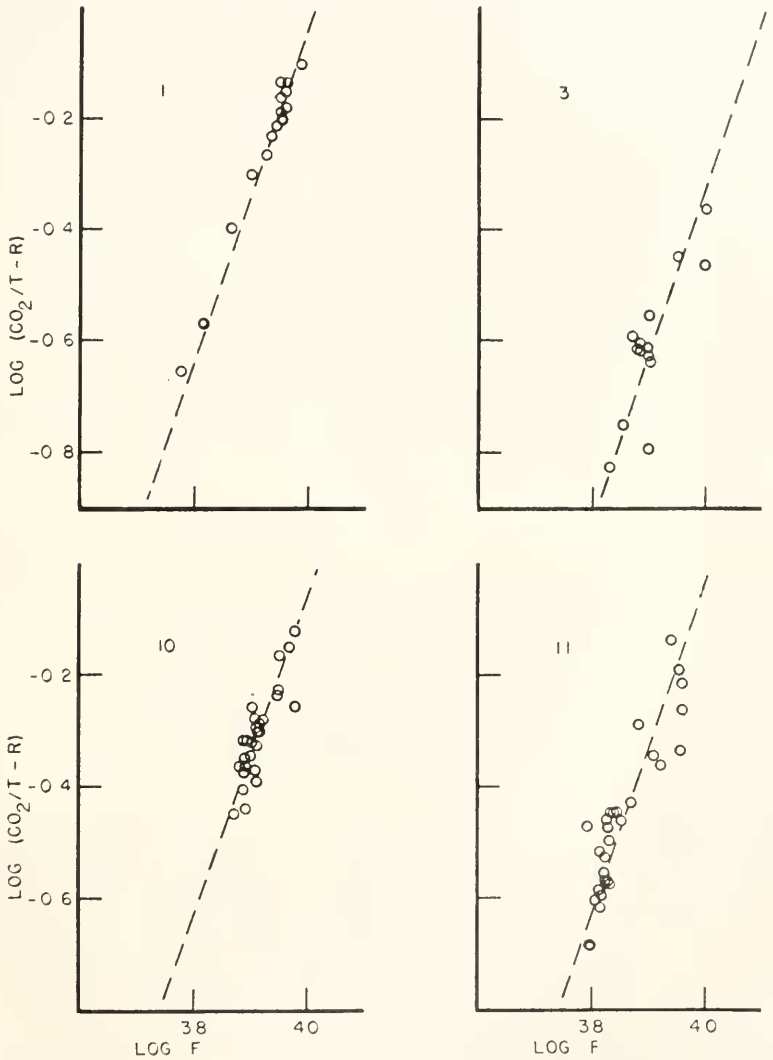

FIGURE 1. CO_2-output as a function of wing frequency in *Drosophila*. Each point represents the average rates during a 5-minute period of flight. The broken lines were calculated to conform to the equation log $(CO_2/t - r) + \log\ k = 3\ \log\ F$, with values chosen for log k as follows: Specimen number 1, 12.03; number 3, 12.32; number 10, 12.05; number 11, 12.03.

The quantity k includes a proportionality factor, plus the efficiency, and probably also a component related to the bodily proportions of the individual insect, in accordance with the findings of Reed *et al.* The values of k used for determining the lines of Figure 1 were calculated for each specimen by averaging the values obtained when the paired observations of $(CO_2/t - r)$ and F were substituted in Equation 1.

Although it is evident from the graphs that there is a fairly close correspondence between performance as predicted by Equation 1 and the actual observations, it is clear also that measurements covering a given 5-minute period may depart rather widely from expectation. These random variations are to be explained as due in part to experimental error, the result chiefly of lag effects in absorption of CO_2 when rapid shifts in the rate of respiration occurred, and may be ascribed also in some measure to alterations in the amplitude of the wing stroke and in the angle of attack. Since the work output per minute would vary with the third power of the amplitude and with the sine of the angle of attack, small variations in these quantities would exert an appreciable effect. Such changes are not infrequently noticed under stroboscopic illumination, but unfortunately no satisfactory technique has been developed for their measurement. The absence, in spite of these irregularities, of any consistent trend away from the linear relationship between F^3 and CO_2-output as lower rates are encountered in the later stages of flight is noteworthy, since it shows that in general the efficiency of flight is the same at this time as when the animals were fresh. This could hardly be the case if any great shift had taken place in the biochemical reactions which deliver the energy for flight; thus these observations give added support to the inference already drawn from the respiratory data: that metabolism of carbohydrate furnishes the energy utilized throughout the entire period.

c. Respiration after flight

For the first 5 or 10 minutes following flight the measurements consistently yielded resting rates of respiration much higher than those found before flight (see Table IV). This is almost certainly an artefact resulting from lag in absorption of CO_2 which had been given off during the flight period, and should not be regarded as evidence for an oxygen debt incurred during activity. With smaller vessels in which the lag in absorption was negligible, it was shown previously that the oxygen debt represented an amount sufficient to sustain flight for only a fraction of a minute, that it was independent of the length of flight, and that it was paid off in 2 minutes after flight had ceased.

During subsequent periods rather variable results were obtained, both as regards the respiratory level and the respiratory quotient. Experimental error was magnified at the lower rate of gaseous exchange, and in addition the activity of the specimens varied from time to time. Nevertheless, the values found for the respiratory quotient were often significantly less than 1, although the rate of oxygen consumption fluctuated for some hours within a range close to the preflight average. These observations accord with other evidence that the carbohydrate reserves are seriously depleted by extended flights.

It is obvious, of course, that flight may be, and normally is terminated by causes other than exhaustion of carbohydrate, and since some individuals were found to be able to fly for over 2 hours, there is a fair presumption that specimens whose performance fell short of this figure may have ceased flying with a considerable store of carbohydrate still in reserve. Some of the higher respiratory quotients recorded

after flight may be accounted for on this basis. The fact that other individuals survived for a number of hours with respiratory quotients of 0.70 or less points to a fundamental difference between the resting metabolism of *Drosophila* and bees, which according to Beutler succumb within a few minutes if they are allowed to fly until their carbohydrate is exhausted. The survival of these flies presents an opportunity for investigating the process of recovery under controlled feeding which could be made to yield valuable information as to the type of substances which can be converted into sources of energy for flight, a phase of the subject concerning which nothing is known at this time.

Summary

1. Continuous volumetric measurements of oxygen consumption and conductimetric measurements of carbon dioxide production were made at 20° C. on individual specimens of *Drosophila americana* and *D. virilis:* (*a*) before flight; (*b*) during flight to exhaustion; and (*c*) after flight. During flight the rate of wing-beat was determined stroboscopically.

2. Before flight, the average results were: CO_2, 31.7 cu.mm. per gm. per min.; O_2, 26.0 cu.mm. per gm. per min.; R. Q., 1.23.

3. During flights lasting from 56 to 154 minutes the rate of respiration was approximately proportional to (wing-beat frequency)^3. The rate of oxygen consumption averaged 14 times and the rate of CO_2-production 11 times the previous resting rate. The R. Q. was essentially 1.

4. Variable rates of respiration were observed after flight; the R. Q. was frequently much less than 1.

5. It is concluded that carbohydrate furnishes the principal and possibly the only source of energy for flight, and that *Drosophila* are able to survive for some hours after their carbohydrate has been exhausted.

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